The Patent Office

INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP9 1RH

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed A M Brewer.

Dated 12 February 1999

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Patents Form 1/77

Patents Act 1977
(Rule 16)

The Patent Office

1/77

12JAN98 E329703-25 D00056
P01/7700 25.00 - 9800487.2

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

RECEIVED BY HAND
9 JAN 1998

09 JAN 1998

The Patent Office
Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference

PWC/AMP/P20678GB

2. Patent application number
(The Patent Office will fill in this part)

9800487.2

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

ORATOL LIMITED
The Old Blue School
Lower Square
Isleworth, Middlesex, TW7 6RL

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

7357927001

4. Title of the invention

THERAPIES

5. Name of your agent *(if you have one)*

KILBURN & STRODE

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

30 John Street
London
WC1N 2DD

Patents ADP number *(if you know it)*

125001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

YES

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	8
Claim(s)	2
Abstract	
Drawing(s)	

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature [signature] Date 9th January 1998

12. Name and daytime telephone number of person to contact in the United Kingdom

Mr. Paul Chapman
Tel: 0171 242 8291

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) For details of the fee and ways to pay please contact the Patent Office.

THERAPIES

This invention relates to therapeutic agents for use in the treatment of mammalian, particularly human, allergic and other hypersensitivity diseases.

Allergies or hypersensitivities are those diseases which occur when the immune system responds 'inappropriately' to foreign antigens (usually environmental macromolecules). In these situations, a normally harmless environmental stimulus, 'allergen', triggers an immune response which, upon re-exposure, is reactivated to generate pathological damage. Immune reactions can be classed as one of 4 types, dependent on the particular components of the immune system which are involved in mediating tissue damage. These are:

Type I: Immediate hypersensitivity/Atopic allergy.

Here the principle immune response to the allergen involves the production of IgE antibodies. Such diseases are by far the most prevalent in humans and are seen as principle targets for new therapeutic approaches. In these diseases IgE binds to cells within the tissues such as mast cells and basophils and the cross-linking of IgE on the cells surface invokes the release of many inflammatory mediators. Examples of such diseases include: Asthma and allergic cough, allergic rhinitis and conjunctivitis, atopic eczema and dermatitis, uticaria, hives, insect bite allergy, dietary allergy (peanut, fish, milk, wheat etc) and certain drug allergies. In many cases the particular allergens are known e.g. DerP1 from house dust mite faeces is a principle allergen in asthma. Other triggers of asthma include particular antigens in pet dander etc.

Type II: Antibody dependent cellular cytotoxicity.

Here the production of antibodies is of a different type, mainly IgG and IgM leads to the destruction of cells of the body to which the antibodies bind. These are relatively unusual but include some allergies to drugs.

Type III: Immune complex disease.

Affects usually the kidneys, skin (rashes) and joints (arthritis). These disorders result from the deposition of antibody, usually IgM or IgG, in the form of antigen-

antibody complexes at these sites. Antibody binds to antigen/allergen and in normal situations the complex is cleared by a variety of tissue cells. However, a number of factors may influence the persistence of the immune complexes, and where these remain in the blood for prolonged periods they can lodge and establish inflammation in the tissues mentioned. These are relatively rare disorders in humans.

Type IV: Delayed type hypersensitivity (DTH).

These conditions do not significantly involve antibody, but instead the prolonged activation of T-lymphocytes. T-cells secrete soluble factors causing tissue damage and enhancing the recruitment and activation of other cell types to the tissues. Incoming cells themselves contribute to the inflammation and tissue damage. These reactions are less common than type I, but include some important diseases e.g. contact sensitivity (allergy usually involving skin rash) to environmental 'contact allergens' such as poison ivy and heavy metals.

WO 95/10301 discloses an immunological tolerance-inducing agent comprising a mucosa-binding molecule linked to a specific tolerogen. In WO 97/02045, it has been shown that autoimmune diseases could be treated using an agent having GM-1 binding activity for instance, when co-administered with an antigen. In contrast to the teaching of WO 95/10301, linkage of the components was shown to be unnecessary. Particular examples of GM-1 binding agents mentioned in WO 97/02045 included Ctx, CtxB, Etx and EtxB.

As used herein, the term "Ctx" refers to the cholera toxin and "CtxB" to the B subunit of the cholera toxin. In other texts, these may sometimes be identified as "CT" or "Ct" and "CTB" or "CtB" respectively. The term "Etx" herein means the *E. coli* heat labile enterotoxin, and "EtxB" is the B subunit of Etx. In other texts, these may sometimes be identified as "LT" or "Lt" and "LTB" or "LtB" respectively.

WO 95/10301 includes mention of the treatment of allergy using a mucosa binding agent coupled to an allergen. Tamura *et al* (Vaccine **15**:225-229 (1997)) take, directly, the protocol of WO 95/10301 and test efficacy in preventing allergy in a

murine model of type I allergy. They saw a significant lowering of IgE levels which are a strong predictor of efficacy. They also cite data not included which shows that EtxB was not effective once IgE levels are established. However, the protocol followed was of course based on coupling the components.

In earlier work (Nashar *et al*, *Proc. Natl. Acad. Sci. USA*. **93**:223-226 (1996); Nashar *et al*, *Int. Immunol.* **8**:731-736 (1996); Williams *et al*, *Proc. Natl. Acad. Sci. USA*. **94**:5290-5295 (1997); and Nashar *et al*, *Immunol.* **91**:572-578 (1997)) we have shown that administration of a GM-1 binding agent can modulate the immune response away from γIFN and toward IL-4 (ie a Th1 to Th2 switch) and that this is effective in treating autoimmune diseases. This is the basis of the disclosure in WO 97/02045. These results would suggest that GM-1 binding agents would therefore not find use in treating allergy since, for instance, type I allergic diseases involve IgE, the production of which is generally accepted to be promoted by IL-4 and down-regulated by γIFN. We have now found that the use of such agents, when co-administered with suitable allergens, can be used as an effective treatment.

Thus, in a first aspect, the present invention provides the use of an agent having GM-1 binding activity, or an agent having an effect on GM-1 mediated intracellular signalling events, but no GM-1 binding activity, in the preparation of a medicament to treat an allergic or other hypersensitive condition, with the proviso that said agent is not coupled with an allergen and/or an antigen. This first aspect of the invention extends to cover the use of all agents having GM1 binding activity, for use in preparing medicaments for the treatment of mammalian allergic or other hypersensitive disease, as well as those agents having an effect on GM-1 mediated intracellular signalling events, and which therefore mimic GM-1 binding agents.

In particular embodiments a GM-1 binding agent such as Ctx, Etx, CtxB or EtxB is employed. In a particularly preferred embodiment EtxB is used. In addition to the wild type EtxB, this preferred aspect of the invention also extends to mutants of EtxB which have GM-1 binding activity as well as to other equivalent proteins, such

as the cholera toxin B subunit (CtxB) and mutants thereof which have GM1 binding activity.

Other therapeutic agents for the treatment of allergic or other hypersensitive disease in accordance with the first aspect of this invention are humanised monoclonal antibodies, which bind GM1. Methods known in the art for identifying and preparing such agents are well known.

In particular the medicament is used for the treatment or prophylaxis of a type I and/or a type IV allergic condition as defined herein and/or other hypersensitivity conditions such as contact hypersensitivity.

The medicament can include one or more allergens/antigens but of course these will not be coupled with the agent.

In a second aspect of the present invention there is provided a method for the treatment or prophylaxis of an allergic or other hypersensitive condition which comprises administering to a subject an effective amount of an agent as defined herein. In this embodiment, the agent is administered to a patient with or without co-administration of an allergen/antigen

In a further aspect the invention provides a vaccine for use in the treatment or prophyalxis of an allergic or other hypersensitive condition which comprises an agent having GM-1 binding activity, or an agent having an effect on GM-1 mediated intracellular signalling events, but no GM-1 binding activity, with the proviso that said agent is not coupled with an allergen and/or an antigen.

In a fourth aspect therefore, the present invention provides a method for the "vaccination" of a mammalian subject against an allergic or other hypersensitive disease, in which an agent as defined herein is co-administered with one or more allergens/antigens associated with said disease

In the above aspect of the invention, the therapeutic agent and the allergen/antigen are, or may be, co-administered to the subject. By this we mean that the site and time of administration of each of the therapeutic agent and the allergen/antigen are such that the necessary modulation of the immune system is achieved. Thus, whilst the therapeutic agent and the allergen/antigen may be administered at the same moment in time and at the same site, there may be advantages in administering the therapeutic agent at a different time and to a different site from the allergen/antigen

Whilst single doses of the therapeutic agent and the antigenic determinant may be satisfactory, multiple doses are contemplated within the scope of this aspect of the invention.

Specific allergic or other hypersensitive diseases which may be treated in accordance with this aspect of the present invention include asthma and allergic cough, allergic rhinitis and conjunctivitis, atopic eczema and dermatitis, uticaria, hives, insect bite allergy, dietary allergy (peanut, fish, milk, wheat etc), drug allergies and other hypersensitivity conditions such as contact hypersensitivity induced by the plant poison ivy. Examples of common allergen sources are outlined in table 1 below

Group	Examples
Airborne	
grass pollens	ragweed, rye, couch, wild oat, timothy, Bermuda, Kentucky blue, mugwort
tree pollens	alder, birch, hazel, beech, Cupressae, oak,olive
moulds	*Aspergillus* spp., *Cladosporium* spp., *Alternaria* spp., Basidospores, Ascomycetes
cereal grains	wheat, rye, oat
animal dander and urine	cat, dog, horse, rabbit, guinea pig, hamster
bird feathers	budgerigar, parrot, pigeon, duck, chicken
house dust mite	*Dermatophagoides pteronyssinus, D.farinae, Euroglyphus maynei*
insects	cockroach, fly, locust, midge
Oral	
foods	seafood, legumes, peanuts, nuts, cereals, dairy products, eggs, fruits, tomatoes, mushrooms, alcoholic beverages, coffee, chocolate
drugs	penicillins, sulphonamides and other antibiotics, sulphasalazine, carbamazepine
Injected	
insects	bee and wasp stings, ant and mosquito bites
drugs	blood products, sera, vaccines, contrast media, drugs (including anti-asthma drugs and antibiotics)

Also provided is a pharmaceutical composition for the treatment of a human allergic or other hypersensitive disease comprising

(i) an agent having GM-1 binding activity; or

(ii) an agent having an effect on GM-1 mediated intracellular signalling events, but no GM-1 binding activity;

with the proviso that the agent is not coupled with an allergen/antigen; and a pharmaceutically acceptable carrier or diluent therefor.

The pharmaceutical composition of this aspect of the invention may be formulated to be delivered by a mucosal route, for example as a nasal spray or aerosol for inhalation or injestable solution, or parenterally in which the composition is formulated in an injectable form, for delivery by, for example, an intravenous, intramuscular or subcutaneous route. Alternatively the formulation may be designed to be delivered by both routes.

The pharmaceutical composition may be formulated together with an appropriate allergen/antigen. Alternatively, a kit may be provided comprising separate compositions for each of the therapeutic agent and the allergen/antigen.

One preferred method of oral delivery uses formulations as described in WO95/13795, WO96/17593 and WO96/17594. These formulations allow macromolecules such as proteins to be solubilised in "oils" for oral delivery. Such formulations therefore allow delivery of the macromolecules to mucosal surfaces in the gut.

When the therapeutic agent of the invention is a protein, such as the EtxB subunit or the CtxB subunit, it may be produced, for use in all aspects of this invention, by a method in which the gene or genes coding for the specific polypeptide chain (or chains) from which the protein is formed, is inserted into a suitable vector and then used to transfect a suitable host. For example, the gene coding for the polypeptide chain from which EtxB assemble may be inserted into, for example, plasmid pMMB68, which is then used to transfect host cells, such as Vibrio sp.60. The

protein is purified and isolated in a manner known per se. Mutant genes expressing active mutant EtxB protein may then be produced by known methods from the wild type gene.

In a further approach, again when the therapeutic agent is a protein, it is possible to deliver such proteins by means of a bacterial delivery system such as that described in WO 93/17117. This system utilises the bacterium *Lactococcus lactis* to deliver proteins, for instance orally or indeed by other mucosal routes such as nasally.

As previously stated, agents having GM-1 binding activity, such as specifically designed humanised monoclonal antibodies, may be designed and produced as outlined above, by methods which are known in the art.

In all aspects of the invention, the agent having GM1 binding activity may also be capable of cross-linking GM1 receptors. EtxB is one such agent which is capable of cross-linking GM1 receptors by virtue of its pentameric form.

In a further aspect the present invention provides a product comprising an agent having GM-1 binding activity, or an agent having an effect on GM-1 mediated intracellular signalling events, but no GM-1 binding activity, in the preparation of a medicament to treat an allergic or other hypersensitive condition, with the proviso that said agent is not coupled with an allergen and/or an antigen, and at least one antigen/allergen as a combined preparation for simultaneous, separate or sequential use.

Preferred features of each aspect of the invention are as for each other aspect, *mutatis mutandis*.

CLAIMS:

1. The use of an agent having GM-1 binding activity, or an agent having an effect on GM-1 mediated intracellular signalling events, but no GM-1 binding activity, in the preparation of a medicament to treat an allergic or other hypersensitive condition, with the proviso that said agent is not coupled with an allergen and/or an antigen.

2. The use as claimed in claim 1 wherein the agent is a GM-1 binding agent such as Ctx, Etx, CtxB or EtxB or a mutant form or derivative thereof.

3. The use as claimed in claim 1 or claim 2 wherein the medicament is for the prophylaxis or treatment of asthma, allergic cough, allergic rhinitis, conjunctivitis, atopic eczema, dermatitis, uticaria, hives, insect bite allergy, dietary allergy (peanut, fish, milk, wheat etc), drug allergies or contact and other hypersensitivities.

4. A method for the treatment or prophylaxis of an allergic or other hypersensitive condition which comprises administering to a subject an effective amount of an agent having GM-1 binding activity, or an agent having an effect on GM-1 mediated intracellular signalling events, but no GM-1 binding activity, with the proviso that said agent is not coupled with an allergen and/or an antigen.

5. A method as claimed in claim 4 wherein the agent is a GM-1 binding agent such as Ctx, Etx, CtxB or EtxB or a mutant form or derivative thereof.

6. A method as claimed in claim 4 or claim 5 wherein the method is for the prophylaxis or treatment of asthma, allergic cough, allergic rhinitis, conjunctivitis, atopic eczema, dermatitis, uticaria, hives, insect bite allergy, dietary allergy (peanut, fish, milk, wheat etc), drug allergies or contact hypersensitivity.

7. A pharmaceutical composition for the treatment of a human allergic and/or hypersensitivity disease comprising

(i) an agent having GM-1 binding activity; or

(ii) an agent having an effect on GM-1 mediated intracellular signalling events, but no GM-1 binding activity;

with the proviso that the agent is not coupled with an allergen/antigen; and a pharmaceutically acceptable carrier or diluent therefor.

8. A product comprising an agent having GM-1 binding activity, or an agent having an effect on GM-1 mediated intracellular signalling events, but no GM-1 binding activity, in the preparation of a medicament to treat an allergic or other hypersensitive condition, with the proviso that said agent is not coupled with an allergen and/or an antigen, and at least one antigen/allergen as a combined preparation for simultaneous, separate or sequential use.